J Med Virol. 2020 Sep;92(9):1649-1656. doi: 10.1002/jmv.25817. Epub 2020 Apr 10.

SARS-CoV-2 spike protein favors ACE2 from Bovidae and Cricetidae

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Affiliations + expand

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the recent COVID-19 public health crisis. Bat is the widely believed original host of SARS-CoV-2. However, its intermediate host before transmitting to humans is not clear. Some studies proposed pangolin, snake, or turtle as the intermediate hosts. Angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV-2, which determines the potential host range for SARS-CoV-2. On the basis of structural information of the complex of human ACE2 and SARS-CoV-2 receptor-binding domain (RBD), we analyzed the affinity to S protein of the 20 key residues in ACE2 from mammal, bird, turtle, and snake. Several ACE2 proteins from Primates, Bovidae, Cricetidae, and Cetacea maintained the majority of key residues in ACE2 for associating with SARS-CoV-2 RBD. The simulated structures indicated that ACE2 proteins from Bovidae and Cricetidae were able to associate with SARS-CoV-2 RBD. We found that nearly half of the key residues in turtle, snake, and bird were changed. The simulated structures showed several key contacts with SARS-CoV-2 RBD in turtle and snake ACE2 were abolished. This study demonstrated that neither snake nor turtle was the intermediate hosts for SARS-CoV-2, which further reinforced the concept that the reptiles are resistant against infection of coronavirus. This study suggested that Bovidae and Cricetidae should be included in the screening of intermediate hosts for SARS-CoV-2.

Review > J Med Virol. 2020 Jul;92(7):770-775. doi: 10.1002/jmv.25887.
Epub 2020 Apr 27.

Hyperglycemia, hydroxychloroquine, and the COVID-19 pandemic

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Free PMC article

Abstract

Coronavirus disease-2019 (COVID-19) infection and its severity can be explained by the concentration of glycosylated severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) viral particles in the lung epithelium, the concentration of glycosylated angiotensin-converting enzyme receptor 2 (ACE2) in the lung epithelium, and the degree and control of the pulmonary immune response to the SARS-CoV-2 spike protein at approximately day 8 to 10 after symptom onset, which may be related to both. Binding of ACE2 by SARS-CoV-2 in COVID-19 also suggests that prolonged uncontrolled hyperglycemia, and not just a history of diabetes mellitus, may be important in the pathogenesis of the disease. It is tempting to consider that the same mechanism acts in COVID-19 as in SARS, where an overactive macrophage M1 inflammatory response, as neutralizing antibodies to the SARS-CoV-2 spike protein form at day 7 to 10, results in acute respiratory distress syndrome (ARDS) in susceptible patients. It also allows consideration of agents, such as hydroxychloroquine, which may interfere with this overly brisk macrophage inflammatory response and perhaps influence the course of the disease, in particular, those that blunt but do not completely abrogate the M1 to M2 balance in macrophage polarization, as well as viral load, which in SARS appears to be temporally related to the onset of ARDS.

› Viruses. 2020 Apr 30;12(5):497. doi: 10.3390/v12050497.

The SARS-CoV-2 Exerts a Distinctive Strategy for Interacting with the ACE2 Human Receptor

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Free PMC article

Abstract

The COVID-19 disease has plagued over 200 countries with over three million cases and has resulted in over 200,000 deaths within 3 months. To gain insight into the high infection rate of the SARS-CoV-2 virus, we compare the interaction between the human ACE2 receptor and the SARS-CoV-2 spike protein with that of other pathogenic coronaviruses using molecular dynamics simulations. SARS-CoV, SARS-CoV-2, and HCoV-NL63 recognize ACE2 as the natural receptor but present a distinct binding interface to ACE2 and a different network of residue-residue contacts. SARS-CoV and SARS-CoV-2 have comparable binding affinities achieved by balancing energetics and dynamics. The SARS-CoV-2-ACE2 complex contains a higher number of contacts, a larger interface area, and decreased interface residue fluctuations relative to the SARS-CoV-ACE2 complex. These findings expose an exceptional evolutionary exploration exerted by coronaviruses toward host recognition. We postulate that the versatility of cell receptor binding strategies has immediate implications for therapeutic strategies.

Review > J Microbiol Immunol Infect. 2020 Jun;53(3):425-435.
doi: 10.1016/j.jmii.2020.04.015. Epub 2020 May 6.

ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome

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Free PMC article

Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has emerged in Chinese people in December 2019 and has currently spread worldwide causing the COVID-19 pandemic with more than 150,000 deaths. In order for a SARS-CoV like virus circulating in wild life for a very long time to infect the index case-patient, a number of conditions must be met, foremost among which is the encounter with humans and the presence in homo sapiens of a cellular receptor allowing the virus to bind. Recently it was shown that the SARS-CoV-2 spike protein, binds to the human angiotensin I converting enzyme 2 (ACE2). This molecule is a peptidase expressed at the surface of lung epithelial cells and other tissues, that regulates the renin-angiotensin-aldosterone system. Humans are not equal with respect to the expression levels of the cellular ACE2. Moreover, ACE2 polymorphisms were recently described in human populations. Here we review the most recent evidence that ACE2 expression and/or polymorphism could influence both the susceptibility of people to SARS-CoV-2 infection and the outcome of the COVID-19 disease. Further exploration of the relationship between the virus, the peptidase function of ACE2 and the levels of angiotensin II in SARS-CoV-2 infected patients should help to better understand the pathophysiology of the disease and the multi-organ failures observed in severe COVID-19 cases, particularly heart failure.

> Int J Antimicrob Agents. 2020 Aug;56(2):106020.
doi: 10.1016/j.ijantimicag.2020.106020. Epub 2020 May 13.

Synergistic antiviral effect of hydroxychloroquine and azithromycin in combination against SARS-CoV-2: What molecular dynamics studies of virus-host interactions reveal

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Affiliations + expand

PMID: 32405156 PMCID: PMC7219429 DOI: 10.1016/j.ijantimicag.2020.106020

Free PMC article

Abstract

The emergence of SARS-coronavirus-2 (SARS-CoV-2) has led to a global pandemic disease referred to as coronavirus disease 19 (COVID-19). Hydroxychloroquine (CLQ-OH)/azithromycin (ATM) combination therapy is currently being tested for the treatment of COVID-19, with promising results. However, the molecular mechanism of action of this combination is not yet established. Using molecular dynamics (MD) simulations, this study shows that the drugs act in synergy to prevent any close contact between the virus and the plasma membrane of host cells. Unexpected molecular similarity is shown between ATM and the sugar moiety of GM1, a lipid raft ganglioside acting as a host attachment cofactor for respiratory viruses. Due to this mimicry, ATM interacts with the ganglioside-binding domain of SARS-CoV-2 spike protein. This binding site shared by ATM and GM1 displays a conserved amino acid triad Q-134/F-135/N-137 located at the tip of the spike protein. CLQ-OH molecules are shown to saturate virus attachment sites on gangliosides in the vicinity of the primary coronavirus receptor, angiotensin-converting enzyme-2 (ACE-2). Taken together, these data show that ATM is directed against the virus, whereas CLQ-OH is directed against cellular attachment cofactors. We conclude that both drugs act as competitive inhibitors of SARS-CoV-2 attachment to the host-cell membrane. This is consistent with a synergistic antiviral mechanism at the plasma membrane level, where therapeutic intervention is likely to be most efficient. This molecular mechanism may explain the beneficial effects of CLQ-OH/ATM combination therapy in patients with COVID-19. Incidentally, the data also indicate that the conserved Q-134/F-135/N-137 triad could be considered as a target for vaccine strategies.

> Int J Infect Dis. 2020 Jul;96:459-460. doi: 10.1016/j.ijid.2020.05.071.
Epub 2020 May 26.

Could the D614G substitution in the SARS-CoV-2 spike (S) protein be associated with higher COVID-19 mortality?

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Free PMC article

Abstract

The increasing number of deaths due to the COVID-19 pandemic has raised serious global concerns. Increased testing capacity and ample intensive care availability could explain lower mortality in some countries compared to others. Nevertheless, it is also plausible that the SARS-CoV-2 mutations giving rise to different phylogenetic clades are responsible for the apparent death rate disparities around the world. Current research literature linking the genetic make-up of SARS-CoV-2 with fatalities is lacking. Here, we suggest that this disparity in fatality rates may be attributed to SARS-CoV-2 evolving mutations and urge the international community to begin addressing the phylogenetic clade classification of SARS-CoV-2 in relation to clinical outcomes.

› ACS Chem Neurosci. 2020 Aug 5;11(15):2361-2369.
doi: 10.1021/acschemneuro.0c00373. Epub 2020 Jul 21.

Considerations around the SARS-CoV-2 Spike Protein with Particular Attention to COVID-19 Brain Infection and Neurological Symptoms

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PMID: 32627524 PMCID: PMC7374936 DOI: 10.1021/acschemneuro.0c00373

Free PMC article

Abstract

Spike protein (S protein) is the virus "key" to infect cells and is able to strongly bind to the human angiotensin-converting enzyme2 (ACE2), as has been reported. In fact, Spike structure and function is known to be highly important for cell infection as well as for entering the brain. Growing evidence indicates that different types of coronaviruses not only affect the respiratory system, but they might also invade the central nervous system (CNS). However, very little evidence has been so far reported on the presence of COVID-19 in the brain, and the potential exploitation, by this virus, of the lung to brain axis to reach neurons has not been completely understood. In this Article, we assessed the SARS-CoV and SARS-CoV-2 Spike protein sequence, structure, and electrostatic potential using computational approaches. Our results showed that the S proteins of SARS-CoV-2 and SARS-CoV are highly similar, sharing a sequence identity of 77%. In addition, we found that the SARS-CoV-2 S protein is slightly more positively charged than that of SARS-CoV since it contains four more positively charged residues and five less negatively charged residues which may lead to an increased affinity to bind to negatively charged regions of other molecules through nonspecific and specific interactions. Analysis the S protein binding to the host ACE2 receptor showed a 30% higher binding energy for SARS-CoV-2 than for the SARS-CoV S protein. These results might be useful for understanding the mechanism of cell entry, blood-brain barrier crossing, and clinical features related to the CNS infection by SARS-CoV-2.

J Hematol Oncol. 2020 Sep 4;13(1):120. doi: 10.1186/s13045-020-00954-7.

SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19

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PMID: 32887634 PMCID: PMC7471641 DOI: 10.1186/s13045-020-00954-7

Free PMC article

Abstract

Background: Critically ill patients diagnosed with COVID-19 may develop a pro-thrombotic state that places them at a dramatically increased lethal risk. Although platelet activation is critical for thrombosis and is responsible for the thrombotic events and cardiovascular complications, the role of platelets in the pathogenesis of COVID-19 remains unclear.

Methods: Using platelets from healthy volunteers, non-COVID-19 and COVID-19 patients, as well as wild-type and hACE2 transgenic mice, we evaluated the changes in platelet and coagulation parameters in COVID-19 patients. We investigated ACE2 expression and direct effect of SARS-CoV-2 virus on platelets by RT-PCR, flow cytometry, Western blot, immunofluorescence, and platelet functional studies in vitro, $FeCl_3$-induced thrombus formation in vivo, and thrombus formation under flow conditions ex vivo.

Results: We demonstrated that COVID-19 patients present with increased mean platelet volume (MPV) and platelet hyperactivity, which correlated with a decrease in overall platelet count. Detectable SARS-CoV-2 RNA in the blood stream was associated with platelet hyperactivity in critically ill patients. Platelets expressed ACE2, a host cell receptor for SARS-CoV-2, and TMPRSS2, a serine protease for Spike protein priming. SARS-CoV-2 and its Spike protein directly enhanced platelet activation such as platelet aggregation, PAC-1 binding, CD62P expression, α granule secretion, dense granule release, platelet spreading, and clot retraction in vitro, and thereby Spike protein enhanced thrombosis formation in wild-type mice transfused with hACE2 transgenic platelets, but this was not observed in animals transfused with wild-type platelets in vivo. Further, we provided evidence suggesting that the MAPK pathway, downstream of ACE2, mediates the potentiating role of SARS-CoV-2 on platelet activation, and that platelet ACE2 expression decreases following SARS-COV-2 stimulation. SARS-CoV-2 and its Spike protein directly stimulated platelets to facilitate the release of coagulation factors, the secretion of inflammatory factors, and the formation of leukocyte-platelet aggregates. Recombinant human ACE2 protein and anti-Spike monoclonal antibody could inhibit SARS-CoV-2 Spike protein-induced platelet activation.

Conclusions: Our findings uncovered a novel function of SARS-CoV-2 on platelet activation via binding of Spike to ACE2. SARS-CoV-2-induced platelet activation may participate in thrombus formation and inflammatory responses in COVID-19 patients.

Cell. 2020 Nov 25;183(5):1340-1353.e16. doi: 10.1016/j.cell.2020.10.001.
Epub 2020 Oct 5.

Imbalance of Regulatory and Cytotoxic SARS-CoV-2-Reactive CD4 $^{+}$ T Cells in COVID-19

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PMID: 33096020 PMCID: PMC7534589 DOI: 10.1016/j.cell.2020.10.001

Free PMC article

Abstract

The contribution of CD4^{+} T cells to protective or pathogenic immune responses to SARS-CoV-2 infection remains unknown. Here, we present single-cell transcriptomic analysis of >100,000 viral antigen-reactive CD4^{+} T cells from 40 COVID-19 patients. In hospitalized patients compared to non-hospitalized patients, we found increased proportions of cytotoxic follicular helper cells and cytotoxic T helper (T_H) cells (CD4-CTLs) responding to SARS-CoV-2 and reduced proportion of SARS-CoV-2-reactive regulatory T cells (T_{REG}). Importantly, in hospitalized COVID-19 patients, a strong cytotoxic T_{FH} response was observed early in the illness, which correlated negatively with antibody levels to SARS-CoV-2 spike protein. Polyfunctional T_H1 and T_H17 cell subsets were underrepresented in the repertoire of SARS-CoV-2-reactive CD4^{+} T cells compared to influenza-reactive CD4^{+} T cells. Together, our analyses provide insights into the gene expression patterns of SARS-CoV-2-reactive CD4^{+} T cells in distinct disease severities.

> Neurobiol Dis. 2020 Dec;146:105131. doi: 10.1016/j.nbd.2020.105131.
Epub 2020 Oct 11.

The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in-vitro models of the human blood-brain barrier

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Free PMC article

Abstract

As researchers across the globe have focused their attention on understanding SARS-CoV-2, the picture that is emerging is that of a virus that has serious effects on the vasculature in multiple organ systems including the cerebral vasculature. Observed effects on the central nervous system include neurological symptoms (headache, nausea, dizziness), fatal microclot formation and in rare cases encephalitis. However, our understanding of how the virus causes these mild to severe neurological symptoms and how the cerebral vasculature is impacted remains unclear. Thus, the results presented in this report explored whether deleterious outcomes from the SARS-CoV-2 viral spike protein on primary human brain microvascular endothelial cells (hBMVECs) could be observed. The spike protein, which plays a key role in receptor recognition, is formed by the S1 subunit containing a receptor binding domain (RBD) and the S2 subunit. First, using postmortem brain tissue, we show that the angiotensin converting enzyme 2 or ACE2 (a known binding target for the SARS-CoV-2 spike protein), is ubiquitously expressed throughout various vessel calibers in the frontal cortex. Moreover, ACE2 expression was upregulated in cases of hypertension and dementia. ACE2 was also detectable in primary hBMVECs maintained under cell culture conditions. Analysis of cell viability revealed that neither the S1, S2 or a truncated form of the S1 containing only the RBD had minimal effects on hBMVEC viability within a 48 h exposure window. Introduction of spike proteins to invitro models of the blood-brain barrier (BBB) showed significant changes to barrier properties. Key to our findings is the demonstration that S1 promotes loss of barrier integrity in an advanced 3D microfluidic model of the human BBB, a platform that more closely resembles the physiological conditions at this CNS interface. Evidence provided suggests that the SARS-CoV-2 spike proteins trigger a pro-inflammatory response on brain endothelial cells that may contribute to an altered state of BBB function. Together, these results are the first to show the direct impact that the SARS-CoV-2 spike protein could have on brain endothelial cells; thereby offering a plausible explanation for the neurological consequences seen in COVID-19 patients.

› Front Immunol. 2020 Oct 16;11:586984. doi: 10.3389/fimmu.2020.586984. eCollection 2020.

Potential Cross-Reactive Immunity to SARS-CoV-2 From Common Human Pathogens and Vaccines

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PMID: 33178220 PMCID: PMC7596387 DOI: 10.3389/fimmu.2020.586984

Free PMC article

Abstract

The recently emerged SARS-CoV-2 causing the ongoing COVID-19 pandemic is particularly virulent in the elderly while children are largely spared. Here, we explored the potential role of cross-reactive immunity acquired from pediatric vaccinations and exposure to common human pathogens in the protection and pathology of COVID-19. To that end, we sought for peptide matches to SARS-CoV-2 (identity ≥ 80%, in at least eight residues) in the proteomes of 25 human pathogens and in vaccine antigens, and subsequently predicted their T and B cell reactivity to identify potential cross-reactive epitopes. We found that viruses subject to pediatric vaccinations do not contain cross-reactive epitopes with SARS-CoV-2, precluding that they can provide any general protection against COVID-19. Likewise, common viruses including rhinovirus, respiratory syncytial virus, influenza virus, and several herpesviruses are also poor or null sources of cross-reactive immunity to SARS-CoV-2, discarding that immunological memory against these viruses can have any general protective or pathological role in COVID-19. In contrast, we found combination vaccines for treating diphtheria, tetanus, and pertussis infectious diseases (DTP vaccine) to be significant sources of potential cross-reactive immunity to SARS-CoV-2. DTP cross-reactive epitopes with SARS-CoV-2 include numerous CD8 and CD4 T cell epitopes with broad population protection coverage and potentially neutralizing B cell epitopes in SARS-CoV-2 Spike protein. Worldwide, children receive several DTP vaccinations, including three-four doses the first year of life and one at 4-6 years of age. Moreover, a low antigenic Tdap dose is also given at ages 9-14. Thereby, children may well be protected from SARS-CoV-2 through cross-reactive immunity elicited by DTP vaccinations, supporting testing in the general population to prevent COVID-19.

> Stem Cell Rev Rep. 2021 Feb;17(1):253-265. doi: 10.1007/s12015-020-10056-z. Epub 2020 Oct 21.

Human Hematopoietic Stem, Progenitor, and Immune Cells Respond Ex Vivo to SARS-CoV-2 Spike Protein

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PMID: 33089452 PMCID: PMC7577648 DOI: 10.1007/s12015-020-10056-z

Free PMC article

Abstract

Despite evidence that SARS-CoV-2 infection is systemic in nature, there is little known about the effects that SARS-CoV-2 infection or exposure has on many host cell types, including primitive and mature hematopoietic cells. The hematopoietic system is responsible for giving rise to the very immune cells that defend against viral infection and is a source of hematopoietic stem cells (HSCs) and progenitor cells (HPCs) which are used for hematopoietic cell transplantation (HCT) to treat hematologic disorders, thus there is a strong need to understand how exposure to the virus may affect hematopoietic cell functions. We examined the expression of ACE2, to which SARS-CoV-2 Spike (S) protein binds to facilitate viral entry, in cord blood derived HSCs/HPCs and in peripheral blood derived immune cell subtypes. ACE2 is expressed in low numbers of immune cells, higher numbers of HPCs, and up to 65% of rigorously defined HSCs. We also examined effects of exposing HSCs/HPCs and immune cells to SARS-CoV-2 S protein ex vivo. HSCs and HPCs expand less effectively and have less functional colony forming capacity when grown with S protein, while peripheral blood monocytes upregulate CD14 expression and show distinct changes in size and granularity. That these effects are induced by recombinant S protein alone and not the infectious viral particle suggests that simple exposure to SARS-CoV-2 may impact HSCs/HPCs and immune cells via S protein interactions with the cells, regardless of whether they can be infected. These data have implications for immune response to SARS-CoV-2 and for HCT. Graphical Abstract • Human HSCs, HPCs, and immune cells express ACE2 on the cell surface, making them potentially susceptible to SARS-CoV-2 infection. • SARS-CoV-2 S protein, which binds to ACE2, induces defects in the colony forming capacity of human HPC and inhibits the expansion of HSC/HPC subpopulations ex vivo. These effects can be at least partially neutralized by treatment with SARS-CoV-2 targeting antibody, recombinant human ACE2, or Angiotensin1-7. • S protein also induces aberrant morphological changes in peripheral blood derived monocytes ex vivo. • Thus, there are many different manners in which SARS-CoV-2 virus may impact the functional hematopoietic system, which has important implications for hematological manifestations of COVID-19 (i.e. thrombocytopenia and lymphopenia), immune response, and hematopoietic stem cell transplant in the era of COVID-19.

J Biol Chem. 2020 Dec 25;295(52):18579-18588. doi: 10.1074/jbc.RA120.015303. Epub 2020 Oct 29.

High affinity binding of SARS-CoV-2 spike protein enhances ACE2 carboxypeptidase activity

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PMID: 33122196 PMCID: PMC7833600 DOI: 10.1074/jbc.RA120.015303

Free PMC article

Abstract

The novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) has emerged to a pandemic and caused global public health crisis. Human angiotensin-converting enzyme 2(ACE2) was identified as the entry receptor for SARS-CoV-2. As a carboxypeptidase, ACE2 cleaves many biological substrates besides angiotensin II to control vasodilatation and vascular permeability. Given the nanomolar high affinity between ACE2 and SARS-CoV-2 spike protein, we investigated how this interaction would affect the enzymatic activity of ACE2. Surprisingly, SARS-CoV-2 trimeric spike protein increased ACE2 proteolytic activity ~3-10 fold against model peptide substrates, such as caspase-1 substrate and Bradykinin-analog. The enhancement in ACE2 enzymatic function was mediated by the binding of SARS-CoV-2 spike RBD domain. These results highlighted the potential for SARS-CoV-2 infection to enhance ACE2 activity, which may be relevant to the cardiovascular symptoms associated with COVID-19.

› Vascul Pharmacol. 2021 Apr;137:106823. doi: 10.1016/j.vph.2020.106823. Epub 2020 Nov 21.

SARS-CoV-2 spike protein-mediated cell signaling in lung vascular cells

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PMID: 33232769 PMCID: PMC7680014 DOI: 10.1016/j.vph.2020.106823

Free PMC article

Abstract

Currently, the world is suffering from the pandemic of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that uses angiotensin-converting enzyme 2 (ACE2) as a receptor to enter the host cells. So far, 60 million people have been infected with SARS-CoV-2, and 1.4 million people have died because of COVID-19 worldwide, causing serious health, economical, and sociological problems. However, the mechanism of the effect of SARS-CoV-2 on human host cells has not been defined. The present study reports that the SARS-CoV-2 spike protein alone without the rest of the viral components is sufficient to elicit cell signaling in lung vascular cells. The treatment of human pulmonary artery smooth muscle cells or human pulmonary artery endothelial cells with recombinant SARS-CoV-2 spike protein S1 subunit (Val16 - Gln690) at 10 ng/ml (0.13 nM) caused an activation of MEK phosphorylation. The activation kinetics was transient with a peak at 10 min. The recombinant protein that contains only the ACE2 receptor-binding domain of the SARS-CoV-2 spike protein S1 subunit (Arg319 - Phe541), on the other hand, did not cause this activation. Consistent with the activation of cell growth signaling in lung vascular cells by the SARS-CoV-2 spike protein, pulmonary vascular walls were found to be thickened in COVID-19 patients. Thus, SARS-CoV-2 spike protein-mediated cell growth signaling may participate in adverse cardiovascular/pulmonary outcomes, and this mechanism may provide new therapeutic targets to combat COVID-19.

› PLoS Pathog. 2020 Dec 7;16(12):e1009128. doi: 10.1371/journal.ppat.1009128. eCollection 2020 Dec.

SARS-CoV-2 spike protein promotes IL-6 trans-signaling by activation of angiotensin II receptor signaling in epithelial cells

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PMID: 33284859 PMCID: PMC7746263 DOI: 10.1371/journal.ppat.1009128

Free PMC article

Abstract

Cytokine storm is suggested as one of the major pathological characteristics of SARS-CoV-2 infection, although the mechanism for initiation of a hyper-inflammatory response, and multi-organ damage from viral infection is poorly understood. In this virus-cell interaction study, we observed that SARS-CoV-2 infection or viral spike protein expression alone inhibited angiotensin converting enzyme-2 (ACE2) receptor protein expression. The spike protein promoted an angiotensin II type 1 receptor (AT1) mediated signaling cascade, induced the transcriptional regulatory molecules NF-κB and AP-1/c-Fos via MAPK activation, and increased IL-6 release. SARS-CoV-2 infected patient sera contained elevated levels of IL-6 and soluble IL-6R. Up-regulated AT1 receptor signaling also influenced the release of extracellular soluble IL-6R by the induction of the ADAM-17 protease. Use of the AT1 receptor antagonist, Candesartan cilexetil, resulted in down-regulation of IL-6/soluble IL-6R release in spike expressing cells. Phosphorylation of STAT3 at the Tyr705 residue plays an important role as a transcriptional inducer for SOCS3 and MCP-1 expression. Further study indicated that inhibition of STAT3 Tyr705 phosphorylation in SARS-CoV-2 infected and viral spike protein expressing epithelial cells did not induce SOCS3 and MCP-1 expression. Introduction of culture supernatant from SARS-CoV-2 spike expressing cells on a model human liver endothelial Cell line (TMNK-1), where transmembrane IL-6R is poorly expressed, resulted in the induction of STAT3 Tyr705 phosphorylation as well as MCP-1 expression. In conclusion, our results indicated that the presence of SARS-CoV-2 spike protein in epithelial cells promotes IL-6 trans-signaling by activation of the AT1 axis to initiate coordination of a hyper-inflammatory response.

› Front Immunol. 2020 Dec 10;11:596684. doi: 10.3389/fimmu.2020.596684. eCollection 2020.

Dynamic SARS-CoV-2-Specific Immunity in Critically Ill Patients With Hypertension

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PMID: 33362779 PMCID: PMC7758245 DOI: 10.3389/fimmu.2020.596684

Free PMC article

Abstract

Background: The current outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses an unprecedented health crisis. The most common chronic illness among patients infected with SARS-CoV-2 is hypertension. Immune dysregulation plays an important role in SARS-CoV-2 infection and in the development of hypertension; however, the dynamic immunological characteristics of COVID-19 patients with hypertension remain largely unclear.

Methods: In total, 258 hypertensive patients infected with SARS-CoV-2 were included in this study. $CD38^+HLA\text{-}DR^+$ and $CD38^+PD\text{-}1^+$ $CD8^+$ T cells, $IFN\gamma^+CD4^+$ and $IFN\gamma^+CD8^+$ T cells, the titers of IgG, IgM, and IgA against SARS-CoV-2 spike protein, and SARS-CoV-2 throat viral loads were measured weekly over 4 weeks after the onset of symptoms. Clinical outcomes were also monitored.

Findings: $CD4^+$ T lymphopenia was observed in 100% of the severe and critical cases. Compared with the surviving patients, the patients with fatal outcomes exhibited high and prolonged expression of $CD38^+HLA\text{-}DR^+$ and $CD38^+PD\text{-}1^+$ on $CD8^+$ T cells, low expression of SARS-CoV-2-specific $IFN\gamma^+CD4^+$ and $IFN\gamma^+CD8^+$ T cells, low titers of IgG, IgM, and IgA against SARS-CoV-2 spike protein, and high SARS-CoV-2 viral load during the illness. In the surviving patients, the viral load was significantly inversely correlated with SARS-CoV-2-specific $IFN\gamma^+CD8^+$and $IFN\gamma^+CD4^+$ T cells, IgG, IgM, and IgA.

Interpretation: T lymphopenia is common in critical or severe COVID-19 cases with hypertension. Prolonged activation and exhaustion of $CD8^+$ T cells were associated with severe disease. The delayed SARS-CoV-2-specific antibody responses may be insufficient for overcoming severe SARS-CoV-2 infection in the absence of SARS-CoV-2-specific cellular responses.

› Cells. 2020 Dec 12;9(12):2676. doi: 10.3390/cells9122676.

IgA2 Antibodies against SARS-CoV-2 Correlate with NET Formation and Fatal Outcome in Severely Diseased COVID-19 Patients

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Affiliations + expand

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Free PMC article

Abstract

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) leads to an adaptive immune response in the host and the formation of anti-SARS-CoV-2 specific antibodies. While IgG responses against SARS-CoV-2 have been characterized quite well, less is known about IgA. IgA2 activates immune cells and induces inflammation and neutrophil extracellular trap (NET) formation which may contribute to organ injury and fatal outcome in SARS-CoV-2-infected patients. SARS-CoV-2 spike protein specific antibody levels were measured in plasma samples of 15 noninfected controls and 82 SARS-CoV-2-infected patients with no or mild symptoms, moderate symptoms (hospitalization) or severe disease (intensive care unit, ICU). Antibody levels were compared to levels of C-reactive protein (CRP) and circulating extracellular DNA (ecDNA) as markers for general inflammation and NET formation, respectively. While levels of SARS-CoV-2-specific IgG were similar in all patient groups, IgA2 antibodies were restricted to severe disease and showed the strongest discrimination between nonfatal and fatal outcome in patients with severe SARS-CoV-2 infection. While anti-SARS-CoV-2 IgG and IgA2 levels correlated with CRP levels in severely diseased patients, only anti-SARS-CoV-2 IgA2 correlated with ecDNA. These data suggest that the formation of anti-SARS-CoV-2 IgA2 during SARS-CoV-2 infection is a marker for more severe disease related to NET formation and poor outcome.

Comparative Study > PLoS Biol. 2020 Dec 21;18(12):e3001016.
doi: 10.1371/journal.pbio.3001016. eCollection 2020 Dec.

The SARS-CoV-2 Spike protein has a broad tropism for mammalian ACE2 proteins

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Affiliations + expand

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Free PMC article

Abstract

SARS Coronavirus 2 (SARS-CoV-2) emerged in late 2019, leading to the Coronavirus Disease 2019 (COVID-19) pandemic that continues to cause significant global mortality in human populations. Given its sequence similarity to SARS-CoV, as well as related coronaviruses circulating in bats, SARS-CoV-2 is thought to have originated in Chiroptera species in China. However, whether the virus spread directly to humans or through an intermediate host is currently unclear, as is the potential for this virus to infect companion animals, livestock, and wildlife that could act as viral reservoirs. Using a combination of surrogate entry assays and live virus, we demonstrate that, in addition to human angiotensin-converting enzyme 2 (ACE2), the Spike glycoprotein of SARS-CoV-2 has a broad host tropism for mammalian ACE2 receptors, despite divergence in the amino acids at the Spike receptor binding site on these proteins. Of the 22 different hosts we investigated, ACE2 proteins from dog, cat, and cattle were the most permissive to SARS-CoV-2, while bat and bird ACE2 proteins were the least efficiently used receptors. The absence of a significant tropism for any of the 3 genetically distinct bat ACE2 proteins we examined indicates that SARS-CoV-2 receptor usage likely shifted during zoonotic transmission from bats into people, possibly in an intermediate reservoir. Comparison of SARS-CoV-2 receptor usage to the related coronaviruses SARS-CoV and RaTG13 identified distinct tropisms, with the 2 human viruses being more closely aligned. Finally, using bioinformatics, structural data, and targeted mutagenesis, we identified amino acid residues within the Spike-ACE2 interface, which may have played a pivotal role in the emergence of SARS-CoV-2 in humans. The apparently broad tropism of SARS-CoV-2 at the point of viral entry confirms the potential risk of infection to a wide range of companion animals, livestock, and wildlife.

› mBio. 2021 Jan 19;12(1):e02940-20. doi: 10.1128/mBio.02940-20.

SARS-CoV-2 Infection Severity Is Linked to Superior Humoral Immunity against the Spike

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Free PMC article

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently causing a global pandemic. The antigen specificity of the antibody response mounted against this novel virus is not understood in detail. Here, we report that subjects with a more severe SARS-CoV-2 infection exhibit a larger antibody response against the spike and nucleocapsid protein and epitope spreading to subdominant viral antigens, such as open reading frame 8 and nonstructural proteins. Subjects with a greater antibody response mounted a larger memory B cell response against the spike, but not the nucleocapsid protein. Additionally, we revealed that antibodies against the spike are still capable of binding the D614G spike mutant and cross-react with the SARS-CoV-1 receptor binding domain. Together, this study reveals that subjects with a more severe SARS-CoV-2 infection exhibit a greater overall antibody response to the spike and nucleocapsid protein and a larger memory B cell response against the spike.**IMPORTANCE** With the ongoing pandemic, it is critical to understand how natural immunity against SARS-CoV-2 and COVID-19 develops. We have identified that subjects with more severe COVID-19 disease mount a more robust and neutralizing antibody response against SARS-CoV-2 spike protein. Subjects who mounted a larger response against the spike also mounted antibody responses against other viral antigens, including the nucleocapsid protein and ORF8. Additionally, this study reveals that subjects with more severe disease mount a larger memory B cell response against the spike. These data suggest that subjects with more severe COVID-19 disease are likely better protected from reinfection with SARS-CoV-2.

> Sci Transl Med. 2021 Jan 27;13(578):eabd6990. doi: 10.1126/scitranslmed.abd6990. Epub 2021 Jan 4.

Stereotypic neutralizing V_H antibodies against SARS-CoV-2 spike protein receptor binding domain in patients with COVID-19 and healthy individuals

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Free PMC article

Abstract

Stereotypic antibody clonotypes exist in healthy individuals and may provide protective immunity against viral infections by neutralization. We observed that 13 of 17 patients with COVID-19 had stereotypic variable heavy chain (V_H) antibody clonotypes directed against the receptor binding domain (RBD) of SARS-CoV-2 spike protein. These antibody clonotypes were composed of immunoglobulin heavy variable 3-53 (*IGHV3-53*) or *IGHV3-66* and immunoglobulin heavy joining 6 (*IGHJ6*) genes. These clonotypes included IgM, IgG3, IgG1, IgA1, IgG2, and IgA2 subtypes and had minimal somatic mutations, which suggested swift class switching after SARS-CoV-2 infection. The different IGHV chains were paired with diverse light chains resulting in binding to the RBD of SARS-CoV-2 spike protein. Human antibodies specific for the RBD can neutralize SARS-CoV-2 by inhibiting entry into host cells. We observed that one of these stereotypic neutralizing antibodies could inhibit viral replication in vitro using a clinical isolate of SARS-CoV-2. We also found that these V_H clonotypes existed in 6 of 10 healthy individuals, with IgM isotypes predominating. These findings suggest that stereotypic clonotypes can develop de novo from naïve B cells and not from memory B cells established from prior exposure to similar viruses. The expeditious and stereotypic expansion of these clonotypes may have occurred in patients infected with SARS-CoV-2 because they were already present.

› Stem Cell Rev Rep. 2021 Feb;17(1):266-277. doi: 10.1007/s12015-020-10010-z.

SARS-CoV-2 Entry Receptor ACE2 Is Expressed on Very Small CD45 $^{-}$ Precursors of Hematopoietic and Endothelial Cells and in Response to Virus Spike Protein Activates the Nlrp3 Inflammasome

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Free PMC article

Abstract

Angiotensin-converting enzyme 2 (ACE2) plays an important role as a member of the renin-angiotensin-aldosterone system (RAAS) in regulating the conversion of angiotensin II (Ang II) into angiotensin (1-7) (Ang [1-7]). But at the same time, while expressed on the surface of human cells, ACE2 is the entry receptor for SARS-CoV-2. Expression of this receptor has been described in several types of cells, including hematopoietic stem cells (HSCs) and endothelial progenitor cells (EPCs), which raises a concern that the virus may infect and damage the stem cell compartment. We demonstrate for the first time that ACE2 and the entry-facilitating transmembrane protease TMPRSS2 are expressed on very small $CD133^{+}CD34^{+}Lin^{-}CD45^{-}$ cells in human umbilical cord blood (UCB), which can be specified into functional HSCs and EPCs. The existence of these cells known as very small embryonic-like stem cells (VSELs) has been confirmed by several laboratories, and some of them may correspond to putative postnatal hemangioblasts. Moreover, we demonstrate for the first time that, in human VSELs and HSCs, the interaction of the ACE2 receptor with the SARS-CoV-2 spike protein activates the Nlrp3 inflammasome, which if hyperactivated may lead to cell death by pyroptosis. Based on this finding, there is a possibility that human VSELs residing in adult tissues could be damaged by SARS-CoV-2, with remote effects on tissue/organ regeneration. We also report that ACE2 is expressed on the surface of murine bone marrow-derived VSELs and HSCs, although it is known that murine cells are not infected by SARS-CoV-2. Finally, human and murine VSELs express several RAAS genes, which sheds new light on the role of these genes in the specification of early-development stem cells. Graphical Abstract •Human VSELs and HSCs express ACE2 receptor for SARS-CoV2 entry. •Interaction of viral spike protein with ACE2 receptor may hyperactivate Nlrp3 inflammasome which induces cell death by pyroptosis. •SARS-CoV2 may also enter cells and eliminate them by cell lysis. •What is not shown since these cells express also Ang II receptor they may hyperactivate Nlrp3 inflammasome in response to Ang II which may induce pyroptosis. Our data indicates that Ang 1-7 may have a protective effect.

› Angew Chem Int Ed Engl. 2021 Mar 22;60(13):7098-7110.
doi: 10.1002/anie.202015639. Epub 2021 Feb 22.

Molecular Simulations suggest Vitamins, Retinoids and Steroids as Ligands of the Free Fatty Acid Pocket of the SARS-CoV-2 Spike Protein*

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Abstract

We investigate binding of linoleate and other potential ligands to the recently discovered fatty acid binding site in the SARS-CoV-2 spike protein, using docking and molecular dynamics simulations. Simulations suggest that linoleate and dexamethasone stabilize the locked spike conformation, thus reducing the opportunity for ACE2 interaction. In contrast, cholesterol may expose the receptor-binding domain by destabilizing the closed structure, preferentially binding to a different site in the hinge region of the open structure. We docked a library of FDA-approved drugs to the fatty acid site using an approach that reproduces the structure of the linoleate complex. Docking identifies steroids (including dexamethasone and vitamin D); retinoids (some known to be active in vitro, and vitamin A); and vitamin K as potential ligands that may stabilize the closed conformation. The SARS-CoV-2 spike fatty acid site may bind a diverse array of ligands, including dietary components, and therefore provides a promising target for therapeutics or prophylaxis.

› Sci Rep. 2021 Mar 10;11(1):5558. doi: 10.1038/s41598-021-84840-3.

Blockade of SARS-CoV-2 spike protein-mediated cell-cell fusion using COVID-19 convalescent plasma

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Free PMC article

Abstract

The recent COVID-19 pandemic poses a serious threat to global public health, thus there is an urgent need to define the molecular mechanisms involved in SARS-CoV-2 spike (S) protein-mediated virus entry that is essential for preventing and/or treating this emerging infectious disease. In this study, we examined the blocking activity of human COVID-19 convalescent plasma by cell-cell fusion assays using SARS-CoV-2-S-transfected 293 T as effector cells and ACE2-expressing 293 T as target cells. We demonstrate that the SARS-CoV-2 S protein exhibits a very high capacity for membrane fusion and is efficient in mediating virus fusion and entry into target cells. Importantly, we find that COVID-19 convalescent plasma with high titers of IgG neutralizing antibodies can block cell-cell fusion and virus entry by interfering with the SARS-CoV-2-S/ACE2 or SARS-CoV-S/ACE2 interactions. These findings suggest that COVID-19 convalescent plasma may not only inhibit SARS-CoV-2-S but also cross-neutralize SARS-CoV-S-mediated membrane fusion and virus entry, supporting its potential as a preventive and/or therapeutic agent against SARS-CoV-2 as well as other SARS-CoV infections.

J Mol Cell Biol. 2021 Mar 10;12(12):916-932. doi: 10.1093/jmcb/mjaa067.

SARS-CoV-2 spike protein binds to bacterial lipopolysaccharide and boosts proinflammatory activity

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Affiliations + expand

PMID: 33295606 PMCID: PMC7799037 DOI: 10.1093/jmcb/mjaa067

Free PMC article

Abstract

There is a link between high lipopolysaccharide (LPS) levels in the blood and the metabolic syndrome, and metabolic syndrome predisposes patients to severe COVID-19. Here, we define an interaction between SARS-CoV-2 spike (S) protein and LPS, leading to aggravated inflammation in vitro and in vivo. Native gel electrophoresis demonstrated that SARS-CoV-2 S protein binds to LPS. Microscale thermophoresis yielded a KD of ~47 nM for the interaction. Computational modeling and all-atom molecular dynamics simulations further substantiated the experimental results, identifying a main LPS-binding site in SARS-CoV-2 S protein. S protein, when combined with low levels of LPS, boosted nuclear factor-kappa B (NF-κB) activation in monocytic THP-1 cells and cytokine responses in human blood and peripheral blood mononuclear cells, respectively. The in vitro inflammatory response was further validated by employing NF-κB reporter mice and in vivo bioimaging. Dynamic light scattering, transmission electron microscopy, and LPS-FITC analyses demonstrated that S protein modulated the aggregation state of LPS, providing a molecular explanation for the observed boosting effect. Taken together, our results provide an interesting molecular link between excessive inflammation during infection with SARS-CoV-2 and comorbidities involving increased levels of bacterial endotoxins.

› Front Immunol. 2021 May 3;12:629102. doi: 10.3389/fimmu.2021.629102. eCollection 2021.

$CD4^+$ T Cells of Prostate Cancer Patients Have Decreased Immune Responses to Antigens Derived From SARS-CoV-2 Spike Glycoprotein

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PMID: 34012431 PMCID: PMC8128251 DOI: 10.3389/fimmu.2021.629102

Free PMC article

Abstract

The adaptive immune response to severe acute respiratory coronavirus 2 (SARS-CoV-2) is important for vaccine development and in the recovery from coronavirus disease 2019 (COVID-19). Men and cancer patients have been reported to be at higher risks of contracting the virus and developing the more severe forms of COVID-19. Prostate cancer (PCa) may be associated with both of these risks. We show that $CD4^+$ T cells of SARS-CoV-2-unexposed patients with hormone-refractory (HR) metastatic PCa had decreased $CD4^+$ T cell immune responses to antigens from SARS-CoV-2 spike glycoprotein but not from the spiked glycoprotein of the 'common cold'-associated human coronavirus 229E (HCoV-229E) as compared with healthy male volunteers who responded comparably to both HCoV-229E- and SARS-CoV-2-derived antigens. Moreover, the HCoV-229E spike glycoprotein antigen-elicited $CD4^+$ T cell immune responses cross-reacted with the SARS-CoV-2 spiked glycoprotein antigens. PCa patients may have impaired responses to the vaccination, and the cross-reactivity can mediate antibody-dependent enhancement (ADE) of COVID-19. These findings highlight the potential for increased vulnerability of PCa patients to COVID-19.

› Biochem Biophys Res Commun. 2021 Jul 5;561:14-18. doi: 10.1016/j.bbrc.2021.05.018. Epub 2021 May 11.

SARS-Cov-2 spike protein fragment 674-685 protects mitochondria from releasing cytochrome c in response to apoptogenic influence

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PMID: 34000512 PMCID: PMC8112323 DOI: 10.1016/j.bbrc.2021.05.018

Free PMC article

Abstract

In spite of numerous studies, many details of SARS-Cov-2 interaction with human cells are still poorly understood. The 674-685 fragment of SARS-Cov-2 spike protein is homologous to the fragment of α-cobratoxin underlying its interaction with α7 nicotinic acetylcholine receptors (nAChRs). The interaction of 674-685 peptide with α7 nAChR has been predicted in silico. In the present paper we confirm this prediction experimentally and investigate the effect of SARS-Cov-2 spike protein peptide on mitochondria, which express α7 nAChRs to regulate apoptosis-related events. We demonstrate that SARS-Cov-2 spike protein peptide 674-685 competes with the antibody against 179-190 fragment of α7 nAChR subunit for the binding to α7-expressing cells and mitochondria and prevents the release of cytochrome c from isolated mitochondria in response to 0.5 mM H_2O_2 but does not protect intact U373 cells against apoptogenic effect of H_2O_2. Our data suggest that the α7 nAChR-binding portion of SARS-Cov-2 spike protein prevents mitochondria-driven apoptosis when the virus is uncoated inside the cell and, therefore, supports the infected cell viability before the virus replication cycle is complete.

› medRxiv. 2021 May 11;2021.05.05.21256686. doi: 10.1101/2021.05.05.21256686.
Preprint

SARS-CoV-2 infection induces autoimmune antibody secretion more in lean than in obese COVID-19 patients

Daniela Frasca, Lisa Reidy, Maria Romero, Alain Diaz, Carolyn Cray, Kristin Kahl, Bonnie B Blomberg

PMID: 34013293 PMCID: PMC8132267 DOI: 10.1101/2021.05.05.21256686

Free PMC article

Abstract

Background/objectives: Obesity decreases the secretion of SARS-CoV-2-specific IgG antibodies in the blood of COVID-19 patients. How obesity impacts the secretion of autoimmune antibodies in COVID-19 patients, however, is not understood. The serum of adult COVID-19 patients contains autoimmune antibodies generated in response to virus-induced tissue damage and cell death leading to the release of intracellular antigens not known to be immunogenic autoantigens. The objective of this study is to evaluate the presence of autoimmune antibodies in COVID-19 patients with obesity.

Subjects/methods: Thirty serum samples from individuals who tested positive for SARS-CoV-2 infection by RT-PCR were collected from inpatient and outpatient settings. Of these, 15 were lean (BMI<25), and 15 were obese (BMI â‰¥30). Control serum samples were from 30 uninfected individuals, age-gender- and BMI-matched, recruited before the current pandemic. Serum IgG antibodies against two autoimmune specificities, as well as against SARS-CoV-2 Spike protein, were measured by ELISA. IgG autoimmune antibodies were specific for malondialdehyde (MDA), a marker of oxidative stress and lipid peroxidation, and for adipocyte-derived protein antigens (AD), markers of virus-induced cell death in the obese AT.

Results: Our results show that SARS-CoV-2 infection induces anti-MDA and anti-AD autoimmune antibodies more in lean than in obese patients as compared to uninfected controls. Serum levels of these autoimmune antibodies, however, are always higher in obese versus lean COVID-19 patients. Moreover, because the autoimmune antibodies found in serum samples of COVID-19 patients have been correlated with serum levels of C-reactive protein (CRP), a general marker of inflammation, we also evaluated the association of anti-MDA and anti-AT antibodies with serum CRP and found a significant association between CRP and autoimmune antibodies in our cohort of lean and obese COVID-19 patients.

Conclusions: Our results highlight the importance of evaluating the quality of the antibody response in COVID-19 patients with obesity, particularly the presence of autoimmune antibodies, and identify biomarkers of self-tolerance breakdown. This is crucial to protect this vulnerable population that is at higher risk of responding poorly to infection with SARS-CoV-2 compared to lean controls.

> PLoS One. 2021 May 12;16(5):e0251585. doi: 10.1371/journal.pone.0251585. eCollection 2021.

Discovery of human ACE2 variants with altered recognition by the SARS-CoV-2 spike protein

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Affiliations + expand

PMID: 33979391 PMCID: PMC8115845 DOI: 10.1371/journal.pone.0251585

Free PMC article

Abstract

Understanding how human ACE2 genetic variants differ in their recognition by SARS-CoV-2 can facilitate the leveraging of ACE2 as an axis for treating and preventing COVID-19. In this work, we experimentally interrogate thousands of ACE2 mutants to identify over one hundred human single-nucleotide variants (SNVs) that are likely to have altered recognition by the virus, and make the complementary discovery that ACE2 residues distant from the spike interface influence the ACE2-spike interaction. These findings illuminate new links between ACE2 sequence and spike recognition, and could find substantial utility in further fundamental research that augments epidemiological analyses and clinical trial design in the contexts of both existing strains of SARS-CoV-2 and novel variants that may arise in the future.

J Phys Chem B. 2021 Jun 3;125(21):5537-5548. doi: 10.1021/acs.jpcb.1c02048. Epub 2021 May 12.

Critical Interactions Between the SARS-CoV-2 Spike Glycoprotein and the Human ACE2 Receptor

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PMID: 33979162 PMCID: PMC8130525 DOI: 10.1021/acs.jpcb.1c02048

Free PMC article

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects human cells by binding its spike (S) glycoproteins to angiotensin-converting enzyme 2 (ACE2) receptors and causes the coronavirus disease 2019 (COVID-19). Therapeutic approaches to prevent SARS-CoV-2 infection are mostly focused on blocking S-ACE2 binding, but critical residues that stabilize this interaction are not well understood. By performing all-atom molecular dynamics (MD) simulations, we identified an extended network of salt bridges, hydrophobic and electrostatic interactions, and hydrogen bonds between the receptor-binding domain (RBD) of the S protein and ACE2. Mutagenesis of these residues on the RBD was not sufficient to destabilize binding but reduced the average work to unbind the S protein from ACE2. In particular, the hydrophobic end of RBD serves as the main anchor site and is the last to unbind from ACE2 under force. We propose that blocking the hydrophobic surface of RBD via neutralizing antibodies could prove to be an effective strategy to inhibit S-ACE2 interactions.

Review > Clin Microbiol Rev. 2021 May 12;34(3):e00299-20.
doi: 10.1128/CMR.00299-20. Print 2021 Jun 16.

Critical Determinants of Cytokine Storm and Type I Interferon Response in COVID-19 Pathogenesis

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PMID: 33980688 PMCID: PMC8142516 DOI: 10.1128/CMR.00299-20

Free PMC article

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), a rapidly evolving pandemic worldwide with at least 68 million COVID-19-positive cases and a mortality rate of about 2.2%, as of 10 December 2020. About 20% of COVID-19 patients exhibit moderate to severe symptoms. Severe COVID-19 manifests as acute respiratory distress syndrome (ARDS) with elevated plasma proinflammatory cytokines, including interleukin 1β (IL-1β), IL-6, tumor necrosis factor α (TNF-α), C-X-C motif chemokine ligand 10 (CXCL10/IP10), macrophage inflammatory protein 1 alpha (MIP-1α), and chemokine (C-C motif) ligand 2 (CCL2), with low levels of interferon type I (IFN-I) in the early stage and elevated levels of IFN-I during the advanced stage of COVID-19. Most of the severe and critically ill COVID-19 patients have had preexisting comorbidities, including hypertension, diabetes, cardiovascular diseases, and respiratory diseases. These conditions are known to perturb the levels of cytokines, chemokines, and angiotensin-converting enzyme 2 (ACE2), an essential receptor involved in SARS-CoV-2 entry into the host cells. ACE2 downregulation during SARS-CoV-2 infection activates the angiotensin II/angiotensin receptor (AT1R)-mediated hypercytokinemia and hyperinflammatory syndrome. However, several SARS-CoV-2 proteins, including open reading frame 3b (ORF3b), ORF6, ORF7, ORF8, and the nucleocapsid (N) protein, can inhibit IFN type I and II (IFN-I and -II) production. Thus, hyperinflammation, in combination with the lack of IFN responses against SARS-CoV-2 early on during infection, makes the patients succumb rapidly to COVID-19. Therefore, therapeutic approaches involving anti-cytokine/anti-cytokine-signaling and IFN therapy would favor the disease prognosis in COVID-19. This review describes critical host and viral factors underpinning the inflammatory "cytokine storm" induction and IFN antagonism during COVID-19 pathogenesis. Therapeutic approaches to reduce hyperinflammation and their limitations are also discussed.

> Zool Res. 2021 May 18;42(3):335-338. doi: 10.24272/j.issn.2095-8137.2021.088.

Particulate matter exposure exacerbates susceptibility to SARS-CoV-2 infection in humanized ACE2 mice

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PMID: 33998180 PMCID: PMC8175951 DOI: 10.24272/j.issn.2095-8137.2021.088

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Abstract in English, Chinese

The global outbreak of coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as of 8 May 2021, has surpassed 150 700 000 infections and 3 279 000 deaths worldwide. Evidence indicates that SARS-CoV-2 RNA can be detected on particulate matter (PM), and COVID-19 cases are correlated with levels of air pollutants. However, the mechanisms of PM involvement in the spread of SARS-CoV-2 remain poorly understood. Here, we found that PM exposure increased the expression level of angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) in several epithelial cells and increased the adsorption of the SARS-CoV-2 spike protein. Instillation of PM in a hACE2 mouse model significantly increased the expression of *ACE2* and *Tmprss2* and viral replication in the lungs. Furthermore, PM exacerbated the pulmonary lesions caused by SARS-CoV-2 infection in the hACE2 mice. In conclusion, our study demonstrated that PM is an epidemiological factor of COVID-19, emphasizing the necessity of wearing anti-PM masks to cope with this global pandemic.

› Cell Metab. 2021 May 18;S1550-4131(21)00230-8. doi: 10.1016/j.cmet.2021.05.013. Online ahead of print.

SARS-CoV-2 infects human pancreatic β cells and elicits β cell impairment

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Free PMC article

Abstract

Emerging evidence points toward an intricate relationship between the pandemic of coronavirus disease 2019 (COVID-19) and diabetes. While preexisting diabetes is associated with severe COVID-19, it is unclear whether COVID-19 severity is a cause or consequence of diabetes. To mechanistically link COVID-19 to diabetes, we tested whether insulin-producing pancreatic β cells can be infected by SARS-CoV-2 and cause β cell depletion. We found that the SARS-CoV-2 receptor, ACE2, and related entry factors (TMPRSS2, NRP1, and TRFC) are expressed in β cells, with selectively high expression of NRP1. We discovered that SARS-CoV-2 infects human pancreatic β cells in patients who succumbed to COVID-19 and selectively infects human islet β cells in vitro. We demonstrated that SARS-CoV-2 infection attenuates pancreatic insulin levels and secretion and induces β cell apoptosis, each rescued by NRP1 inhibition. Phosphoproteomic pathway analysis of infected islets indicates apoptotic β cell signaling, similar to that observed in type 1 diabetes (T1D). In summary, our study shows SARS-CoV-2 can directly induce β cell killing.

› Int J Mol Sci. 2021 May 19;22(10):5336. doi: 10.3390/ijms22105336.

Contribution of Syndecans to the Cellular Entry of SARS-CoV-2

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Affiliations + expand

PMID: 34069441 PMCID: PMC8159090 DOI: 10.3390/ijms22105336

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel emerging pathogen causing an unprecedented pandemic in 21st century medicine. Due to the significant health and economic burden of the current SARS-CoV-2 outbreak, there is a huge unmet medical need for novel interventions effectively blocking SARS-CoV-2 infection. Unknown details of SARS-CoV-2 cellular biology hamper the development of potent and highly specific SARS-CoV-2 therapeutics. Angiotensin-converting enzyme-2 (ACE2) has been reported to be the primary receptor for SARS-CoV-2 cellular entry. However, emerging scientific evidence suggests the involvement of additional membrane proteins, such as heparan sulfate proteoglycans, in SARS-CoV-2 internalization. Here, we report that syndecans, the evolutionarily conserved family of transmembrane proteoglycans, facilitate the cellular entry of SARS-CoV-2. Among syndecans, the lung abundant syndecan-4 was the most efficient in mediating SARS-CoV-2 uptake. The S1 subunit of the SARS-CoV-2 spike protein plays a dominant role in the virus's interactions with syndecans. Besides the polyanionic heparan sulfate chains, other parts of the syndecan ectodomain, such as the cell-binding domain, also contribute to the interaction with SARS-CoV-2. During virus internalization, syndecans colocalize with ACE2, suggesting a jointly shared internalization pathway. Both ACE2 and syndecan inhibitors exhibited significant efficacy in reducing the cellular entry of SARS-CoV-2, thus supporting the complex nature of internalization. Data obtained on syndecan specific in vitro assays present syndecans as novel cellular targets of SARS-CoV-2 and offer molecularly precise yet simple strategies to overcome the complex nature of SARS-CoV-2 infection.

› Sci Rep. 2021 May 21;11(1):10696. doi: 10.1038/s41598-021-90278-4.

SARS-CoV-2 and other human coronavirus show genome patterns previously associated to reduced viral recognition and altered immune response

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Affiliations + expand

PMID: 34021237 PMCID: PMC8139983 DOI: 10.1038/s41598-021-90278-4

Free PMC article

Abstract

A new pandemic caused by the betacoronavirus SARS-CoV-2 originated in China in late 2019. Although often asymptomatic, a relevant percentage of affected people can develop severe pneumonia. Initial evidence suggests that dysregulation of the immune response could contribute to the pathogenesis, as previously demonstrated for SARS-CoV. The presence of genome composition features involved in delaying viral recognition is herein investigated for human coronaviruses (HCoVs), with a special emphasis on SARS-CoV-2. A broad collection of HCoVs polyprotein, envelope, matrix, nucleocapsid and spike coding sequences was downloaded and several statistics representative of genome composition and codon bias were investigated. A model able to evaluate and test the presence of a significant under- or over-representation of dinucleotide pairs while accounting for the underlying codon bias and protein sequence was also implemented. The study revealed the significant under-representation of CpG dinucleotide pair in all HcoV, but especially in SARS-CoV and even more in SARS-CoV-2. The presence of forces acting to minimize CpG content was confirmed by relative synonymous codon usage pattern. Codons containing the CpG pair were severely under-represented, primarily in the polyprotein and spike coding sequences of SARS-CoV-2. Additionally, a significant under-representation of the TpA pair was observed in the N and S region of SARS-CoV and SARS-CoV-2. Increasing experimental evidence has proven that CpG and TpA are targeted by innate antiviral host defences, contributing both to RNA degradation and RIG-1 mediated interferon production. The low content of these dinucleotides could contribute to a delayed interferon production, dysregulated immune response, higher viral replication and poor outcome. Significantly, the RIG-1 signalling pathway was proven to be defective in elderlies, suggesting a likely interaction between limited viral recognition and lower responsiveness in interferon production that could justify the higher disease severity and mortality in older patients.

› Nature. 2021 May 24. doi: 10.1038/s41586-021-03647-4. Online ahead of print.

SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans

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PMID: 34030176 DOI: 10.1038/s41586-021-03647-4

Abstract

Long-lived bone marrow plasma cells (BMPCs) are a persistent and essential source of protective antibodies[1-7]. Individuals who have recovered from COVID-19 have a substantially lower risk of reinfection with SARS-CoV-2[8-10]. Nonetheless, it has been reported that levels of anti-SARS-CoV-2 serum antibodies decrease rapidly in the first few months after infection, raising concerns that long-lived BMPCs may not be generated and humoral immunity against SARS-CoV-2 may be short-lived[11-13]. Here we show that in convalescent individuals who had experienced mild SARS-CoV-2 infections (n = 77), levels of serum anti-SARS-CoV-2 spike protein (S) antibodies declined rapidly in the first 4 months after infection and then more gradually over the following 7 months, remaining detectable at least 11 months after infection. Anti-S antibody titres correlated with the frequency of S-specific plasma cells in bone marrow aspirates from 18 individuals who had recovered from COVID-19 at 7 to 8 months after infection. S-specific BMPCs were not detected in aspirates from 11 healthy individuals with no history of SARS-CoV-2 infection. We show that S-binding BMPCs are quiescent, which suggests that they are part of a stable compartment. Consistently, circulating resting memory B cells directed against SARS-CoV-2 S were detected in the convalescent individuals. Overall, our results indicate that mild infection with SARS-CoV-2 induces robust antigen-specific, long-lived humoral immune memory in humans.

› Sci Immunol. 2021 May 25;6(59):eabj1750. doi: 10.1126/sciimmunol.abj1750.

SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees

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PMID: 34035118 DOI: 10.1126/sciimmunol.abj1750

Abstract

The emergence of SARS-CoV-2 variants harboring mutations in the spike (S) protein has raised concern about potential immune escape. Here, we studied humoral and cellular immune responses to wild type SARS-CoV-2 and the B.1.1.7 and B.1.351 variants of concern in a cohort of 121 BNT162b2 mRNA-vaccinated health care workers (HCW). Twenty-three HCW recovered from mild COVID-19 disease and exhibited a recall response with high levels of SARS-CoV-2-specific functional antibodies and virus-specific T cells after a single vaccination. Specific immune responses were also detected in seronegative HCW after one vaccination, but a second dose was required to reach high levels of functional antibodies and cellular immune responses in all individuals. Vaccination-induced antibodies cross-neutralized the variants B.1.1.7 and B.1.351, but the neutralizing capacity and Fc-mediated functionality against B.1.351 was consistently 2- to 4-fold lower than to the homologous virus. In addition, peripheral blood mononuclear cells were stimulated with peptide pools spanning the mutated S regions of B.1.1.7 and B.1.351 to detect cross-reactivity of SARS-CoV-2-specific T cells with variants. Importantly, we observed no differences in $CD4^+$ T-cell activation in response to variant antigens, indicating that the B.1.1.7 and B.1.351 S proteins do not escape T-cell-mediated immunity elicited by the wild type S protein. In conclusion, this study shows that some variants can partially escape humoral immunity induced by SARS-CoV-2 infection or BNT162b2 vaccination, but S-specific $CD4^+$ T-cell activation is not affected by the mutations in the B.1.1.7 and B.1.351 variants.

› Diabet Med. 2021 May 27;e14608. doi: 10.1111/dme.14608. Online ahead of print.

Severe acute respiratory syndrome coronavirus 2 as a potential cause of type 1 diabetes facilitated by spike protein receptor binding domain attachment to human islet cells: An illustrative case study and experimental data

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PMID: 34043837 DOI: 10.1111/dme.14608

Abstract

Aims: Aim of this study is to report severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, responsible for coronavirus disease 2019 (COVID-19), as a possible cause for type 1 diabetes by providing an illustrative clinical case of a man aged 45 years presenting with antibody-negative diabetic ketoacidosis post-recovery from COVID-19 pneumonia and to explore the potential for SARS-CoV-2 to adhere to human islet cells.

Methods: Explanted human islet cells from three independent solid organ donors were incubated with the SARS-CoV-2 spike protein receptor biding domain (RBD) fused to a green fluorescent protein (GFP) or a control-GFP, with differential adherence established by flow cytometry.

Results: Flow cytometry revealed dose-dependent specific binding of RBD-GFP to islet cells when compared to control-GFP.

Conclusions: Although a causal basis remains to be established, our case and in vitro data highlight a potential mechanism by which SARS-CoV-2 infection may result in antibody-negative type 1 diabetes.

Review > Vaccines (Basel). 2021 May 27;9(6):559. doi: 10.3390/vaccines9060559.

Thrombotic Thrombocytopenia after COVID-19 Vaccination: In Search of the Underlying Mechanism

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Affiliations + expand

PMID: 34071883 PMCID: PMC8227748 DOI: 10.3390/vaccines9060559

Free PMC article

Abstract

The rollout of COVID-19 vaccines brings hope for successful pandemic mitigation and getting the transmission of SARS-CoV-2 under control. The vaccines authorized in Europe displayed a good safety profile in the clinical trials. However, during their post-authorization use, unusual thrombotic events associated with thrombocytopenia have rarely been reported for vector vaccines. This led to the temporary suspension of the AZD1222 vaccine (Oxford/AstraZeneca) in various European countries and the Ad26.COV2 vaccine (Janssen/Johnson&Johnson) in the United States, with regulatory bodies launching investigations into potential causal associations. The thromboembolic reactions were also rarely reported after mRNA vaccines. The exact cause of these adverse effects remains to be elucidated. The present paper outlines the hypotheses on the mechanisms behind the very rare thrombotic thrombocytopenia reported after the COVID-19 vaccination, along with currently existing evidence and future research prospects. The following are discussed: (i) the role of antibodies against platelet factor 4 (PF4), (ii) the direct interaction between adenoviral vector and platelets, (iii) the cross-reactivity of antibodies against SARS-CoV-2 spike protein with PF4, (iv) cross-reactivity of anti-adenovirus antibodies and PF4, (v) interaction between spike protein and platelets, (vi) the platelet expression of spike protein and subsequent immune response, and (vii) the platelet expression of other adenoviral proteins and subsequent reactions. It is also plausible that thrombotic thrombocytopenia after the COVID-19 vaccine is multifactorial. The elucidation of the causes of these adverse events is pivotal in taking precautionary measures and managing vaccine hesitancy. It needs to be stressed, however, that the reported cases are currently sporadic and that the benefits of COVID-19 vaccines vastly outweigh their potential risks.

› Blood. 2021 Jun 1;blood.2021010685. doi: 10.1182/blood.2021010685.
Online ahead of print.

SARS-CoV-2 infection induces the activation of tissue factor-mediated coagulation by activation of acid sphingomyelinase

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PMID: 34075401 PMCID: PMC8172270 DOI: 10.1182/blood.2021010685

Free PMC article

Abstract

SARS-CoV-2 infection is associated with the hypercoagulable state. Tissue factor (TF) is the primary cellular initiator of coagulation. Most of the TF expressed on cell surfaces remains cryptic. Sphingomyelin (SM) is responsible for maintaining TF in the encrypted state, and hydrolysis of SM by acid sphingomyelinase (ASMase) increases TF activity. ASMase was shown to play a role in virus infection biology. In the present study, we investigated the role of ASMase in SARS-CoV-2 infection-induced TF procoagulant activity. Infection of human monocyte-derived macrophages (MDMs) with SARs-CoV-2 spike protein pseudovirus (SARS-CoV-2-SP-PV) markedly increased TF procoagulant activity at the cell surface and released TF+ extracellular vesicles (EVs). The pseudovirus infection did not increase either TF protein expression or phosphatidylserine externalization. SARS-CoV-2-SP-PV infection induced the translocation of ASMase to the outer leaflet of the plasma membrane, which led to the hydrolysis of SM in the membrane. Pharmacological inhibitors or genetic silencing of ASMase attenuated SARS-CoV-2-SP-PV-induced increased TF activity. Inhibition of SARS-CoV-2 receptor, angiotensin-converting enzyme-2, attenuated SARS-CoV-2-SP-PV-induced increased TF activity. Overall, our data suggest that SARS-CoV-2 infection activates the coagulation by decrypting TF through activation of ASMase. Our data suggest that the FDA-approved functional inhibitors of ASMase may help treat hypercoagulability in COVID-19 patients.

› Am J Surg Pathol. 2021 Jun 3. doi: 10.1097/PAS.0000000000001755.
Online ahead of print.

Intestinal Abnormalities in Patients With SARS-CoV-2 Infection: Histopathologic Changes Reflect Mechanisms of Disease

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PMID: 34081038 DOI: 10.1097/PAS.0000000000001755

Abstract

Approximately 20% of patients with symptomatic syndrome-associated coronavirus-2 (SARS-CoV-2) infection have gastrointestinal bleeding and/or diarrhea. Most are managed without endoscopic evaluation because the risk of practitioner infection outweighs the value of biopsy analysis unless symptoms are life-threatening. As a result, much of what is known about the gastrointestinal manifestations of coronavirus disease-2019 (COVID-19) has been gleaned from surgical and autopsy cases that suffer from extensive ischemic injury and/or poor preservation. There are no detailed reports describing any other gastrointestinal effects of SARS-CoV-2 even though >3,000,000 people have died from COVID-19 worldwide. The purpose of this study is to report the intestinal findings related to SARS-CoV-2 infection by way of a small case series including one with evidence of direct viral cytopathic effect and 2 with secondary injury attributed to viral infection. Infection can be confirmed by immunohistochemical stains directed against SARS-CoV-2 spike protein, in situ hybridization for spike protein-encoding RNA, and ultrastructural visualization of viruses within the epithelium. It induces cytoplasmic blebs and tufted epithelial cells without inflammation and may not cause symptoms. In contrast, SARS-CoV-2 infection can cause gastrointestinal symptoms after the virus is no longer detected, reflecting systemic activation of cytokine and complement cascades rather than direct viral injury. Reversible mucosal ischemia features microvascular injury with hemorrhage, small vessel thrombosis, and platelet-rich thrombi. Systemic cytokine elaboration and dysbiosis likely explain epithelial cell injury that accompanies diarrheal symptoms. These observations are consistent with clinical and in vitro data and contribute to our understanding of the protean manifestations of COVID-19.

› Front Immunol. 2021 Jun 4;12:658428. doi: 10.3389/fimmu.2021.658428. eCollection 2021.

SARS-CoV-2 Spike Protein Suppresses ACE2 and Type I Interferon Expression in Primary Cells From Macaque Lung Bronchoalveolar Lavage

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PMID: 34149696 PMCID: PMC8213020 DOI: 10.3389/fimmu.2021.658428

Free PMC article

Abstract

SARS-CoV-2 virus causes upper and lower respiratory diseases including pneumonia, and in some cases, leads to lethal pulmonary failure. Angiotensin converting enzyme-2 (ACE2), the receptor for cellular entry of SARS-CoV-2 virus, has been shown to protect against severe acute lung failure. Here, we provide evidence that SARS-CoV-2 spike protein S1 reduced the mRNA expression of ACE2 and type I interferons in primary cells of lung bronchoalveolar lavage (BAL) from naïve rhesus macaques. The expression levels of ACE2 and type I interferons were also found to be correlated with each other, consistent with the recent finding that ACE2 is an interferon-inducible gene. Furthermore, induction of ACE2 and type I interferons by poly I:C, an interferon inducer, was suppressed by S1 protein in primary cells of BAL. These observations suggest that the downregulation of ACE2 and type I interferons induced by S1 protein may directly contribute to SARS-CoV-2-associated lung diseases.

Editorial > J Biol Regul Homeost Agents. 2021 Jun 8;35(3).
doi: 10.23812/THEO_EDIT_3_21. Online ahead of print.

Be aware of SARS-CoV-2 spike protein: There is more than meets the eye

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PMID: 34100279 DOI: 10.23812/THEO_EDIT_3_21

Abstract

The COVID-19 pandemic necessitated the rapid production of vaccines aimed at the production of neutralizing antibodies against the COVID-19 spike protein required for the corona virus binding to target cells. The best well-known vaccines have utilized either mRNA or an adenovirus vector to direct human cells to produce the spike protein against which the body produces mostly neutralizing antibodies. However, recent reports have raised some skepticism as to the biologic actions of the spike protein and the types of antibodies produced. One paper reported that certain antibodies in the blood of infected patients appear to change the shape of the spike protein so as to make it more likely to bind to cells, while other papers showed that the spike protein by itself (without being part of the corona virus) can damage endothelial cells and disrupt the blood-brain barrier. These findings may be even more relevant to the pathogenesis of long-COVID syndrome that may affect as many as 50% of those infected with SARS-CoV-2. In COVID-19, a response to oxidative stress is required by increasing anti-oxidant enzymes. In this regard, it is known that polyphenols are natural anti-oxidants with multiple health effects. Hence, there are even more reasons to intervene with the use of anti-oxidant compounds, such as luteolin, in addition to available vaccines and anti-inflammatory drugs to prevent the harmful actions of the spike protein.

› Sci Rep. 2021 Jun 9;11(1):12157. doi: 10.1038/s41598-021-91231-1.

Endothelial glycocalyx shields the interaction of SARS-CoV-2 spike protein with ACE2 receptors

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PMID: 34108510 PMCID: PMC8190434 DOI: 10.1038/s41598-021-91231-1

Free PMC article

Abstract

Endothelial cells (ECs) play a crucial role in the development and propagation of the severe COVID-19 stage as well as multiorgan dysfunction. It remains, however, controversial whether COVID-19-induced endothelial injury is caused directly by the infection of ECs with SARS-CoV-2 or via indirect mechanisms. One of the major concerns is raised by the contradictory data supporting or denying the presence of ACE2, the SARS-CoV-2 binding receptor, on the EC surface. Here, we show that primary human pulmonary artery ECs possess ACE2 capable of interaction with the viral Spike protein (S-protein) and demonstrate the crucial role of the endothelial glycocalyx in the regulation of the S-protein binding to ACE2 on ECs. Using force spectroscopy method, we directly measured ACE2- and glycocalyx-dependent adhesive forces between S-protein and ECs and characterized the nanomechanical parameters of the cells exposed to S-protein. We revealed that the intact glycocalyx strongly binds S-protein but screens its interaction with ACE2. Reduction of glycocalyx layer exposes ACE2 receptors and promotes their interaction with S-protein. These results indicate that the susceptibility of ECs to COVID-19 infection may depend on the glycocalyx condition.

› Front Mol Biosci. 2021 Jun 11;8:649575. doi: 10.3389/fmolb.2021.649575. eCollection 2021.

Heparan Sulfate Facilitates Spike Protein-Mediated SARS-CoV-2 Host Cell Invasion and Contributes to Increased Infection of SARS-CoV-2 G614 Mutant and in Lung Cancer

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PMID: 34179075 PMCID: PMC8231436 DOI: 10.3389/fmolb.2021.649575

Abstract

The severe acute respiratory syndrome (SARS)-like coronavirus disease (COVID-19) is caused by SARS-CoV-2 and has been a serious threat to global public health with limited treatment. Cellular heparan sulfate (HS) has been found to bind SARS-CoV-2 spike protein (SV2-S) and co-operate with cell surface receptor angiotensin-converting enzyme 2 (ACE2) to mediate SARS-CoV-2 infection of host cells. In this study, we determined that host cell surface SV2-S binding depends on and correlates with host cell surface HS expression. This binding is required for SARS-Cov-2 virus to infect host cells and can be blocked by heparin lyase, HS antagonist surfen, heparin, and heparin derivatives. The binding of heparin/HS to SV2-S is mainly determined by its overall sulfation with potential, minor contribution of specific SV2-S binding motifs. The higher binding affinity of SV2-S G614 mutant to heparin and upregulated HS expression may be one of the mechanisms underlying the higher infectivity of the SARS-CoV-2 G614 variant and the high vulnerability of lung cancer patients to SARS-CoV-2 infection, respectively. The higher host cell infection by SARS-CoV-2 G614 variant pseudovirus and the increased infection caused by upregulated HS expression both can be effectively blocked by heparin lyase and heparin, and possibly surfen and heparin derivatives too. Our findings support blocking HS-SV2-S interaction may provide one addition to achieve effective prevention and/treatment of COVID-19.

> Cell Rep. 2021 Jun 11;109320. doi: 10.1016/j.celrep.2021.109320.
Online ahead of print.

Memory B cells targeting SARS-CoV-2 spike protein and their dependence on CD4 $^{+}$ T cell help

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PMID: 34146478 PMCID: PMC8192958 DOI: 10.1016/j.celrep.2021.109320

Free PMC article

Abstract

Memory B cells seem to be more durable than antibodies and thus crucial for the long-term immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Here we investigate SARS-CoV-2 spike-specific memory B cells and their dependence on $CD4^{+}$ T cell help in different settings of coronavirus disease 2019 (COVID-19). Compared with severely ill individuals, those who recovered from mild COVID-19 develop fewer but functionally superior spike-specific memory B cells. Generation and affinity maturation of these cells is best associated with $IL\text{-}21^{+}CD4^{+}$ T cells in recovered individuals and $CD40L^{+}CD4^{+}$ T cells in severely ill individuals. The increased activation and exhaustion of memory B cells observed during COVID-19 correlates with $CD4^{+}$ T cell functions. Intriguingly, $CD4^{+}$ T cells recognizing membrane protein show a stronger association with spike-specific memory B cells than those recognizing spike or nucleocapsid proteins. Overall, we identify $CD4^{+}$ T cell subsets associated with the generation of B cell memory during SARS-CoV-2 infection.

> Front Cardiovasc Med. 2021 Jun 11;8:687783. doi: 10.3389/fcvm.2021.687783. eCollection 2021.

SARS-CoV-2 Spike Protein Induces Degradation of Junctional Proteins That Maintain Endothelial Barrier Integrity

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Affiliations + expand

PMID: 34179146 PMCID: PMC8225996 DOI: 10.3389/fcvm.2021.687783

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses the Angiotensin converting enzyme 2 (ACE2) receptor present on the cell surface to enter cells. Angiotensin converting enzyme 2 is present in many cell types including endothelial cells, where it functions to protect against oxidative damage. There is growing evidence to suggest that coronavirus disease (COVID-19) patients exhibit a wide range of post-recovery symptoms and shows signs related to cardiovascular and specifically, endothelial damage. We hypothesized that these vascular symptoms might be associated with disrupted endothelial barrier integrity. This was investigated *in vitro* using endothelial cell culture and recombinant SARS-CoV-2 spike protein S1 Receptor-Binding Domain (Spike). Mouse brain microvascular endothelial cells from normal (C57BL/6 mice) and diabetic (db/db) mice were used. An endothelial transwell permeability assay revealed increased permeability in diabetic cells as well as after Spike treatment. The expression of VE-Cadherin, an endothelial adherens junction protein, JAM-A, a tight junctional protein, Connexin-43, a gap junctional protein, and PECAM-1, were all decreased significantly after Spike treatment in control and to a greater extent, in diabetic cells. In control cells, Spike treatment increased association of endothelial junctional proteins with Rab5a, a mediator of the endocytic trafficking compartment. In cerebral arteries isolated from control and diabetic animals, Spike protein had a greater effect in downregulating expression of endothelial junctional proteins in arteries from diabetic animals than from control animals. In conclusion, these experiments reveal that Spike-induced degradation of endothelial junctional proteins affects endothelial barrier function and is the likely cause of vascular damage observed in COVID-19 affected individuals.

J Colloid Interface Sci. 2021 Jun 12;602:732-739. doi: 10.1016/j.jcis.2021.06.056. Online ahead of print.

SARS-CoV-2 spike protein removes lipids from model membranes and interferes with the capacity of high density lipoprotein to exchange lipids

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PMID: 34157514 PMCID: PMC8195693 DOI: 10.1016/j.jcis.2021.06.056

Free PMC article

Abstract

Cholesterol has been shown to affect the extent of coronavirus binding and fusion to cellular membranes. The severity of Covid-19 infection is also known to be correlated with lipid disorders. Furthermore, the levels of both serum cholesterol and high-density lipoprotein (HDL) decrease with Covid-19 severity, with normal levels resuming once the infection has passed. Here we demonstrate that the SARS-CoV-2 spike (S) protein interferes with the function of lipoproteins, and that this is dependent on cholesterol. In particular, the ability of HDL to exchange lipids from model cellular membranes is altered when co-incubated with the spike protein. Additionally, the S protein removes lipids and cholesterol from model membranes. We propose that the S protein affects HDL function by removing lipids from it and remodelling its composition/structure.

> Sci Rep. 2021 Jun 14;11(1):12448. doi: 10.1038/s41598-021-91746-7.

Glycan reactive anti-HIV-1 antibodies bind the SARS-CoV-2 spike protein but do not block viral entry

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PMID: 34127709 PMCID: PMC8203731 DOI: 10.1038/s41598-021-91746-7

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Abstract

The SARS-CoV-2 spike glycoprotein is a focal point for vaccine immunogen and therapeutic antibody design, and also serves as a critical antigen in the evaluation of immune responses to COVID-19. A common feature amongst enveloped viruses such as SARS-CoV-2 and HIV-1 is the propensity for displaying host-derived glycans on entry spike proteins. Similarly displayed glycosylation motifs can serve as the basis for glyco-epitope mediated cross-reactivity by antibodies, which can have important implications on virus neutralization, antibody-dependent enhancement (ADE) of infection, and the interpretation of antibody titers in serological assays. From a panel of nine anti-HIV-1 gp120 reactive antibodies, we selected two (PGT126 and PGT128) that displayed high levels of cross-reactivity with the SARS-CoV-2 spike. We report that these antibodies are incapable of neutralizing pseudoviruses expressing SARS-CoV-2 spike proteins and are unlikely to mediate ADE via FcγRII receptor engagement. Nevertheless, ELISA and other immunoreactivity experiments demonstrate these antibodies are capable of binding the SARS-CoV-2 spike in a glycan-dependent manner. These results contribute to the growing literature surrounding SARS-CoV-2 S cross-reactivity, as we demonstrate the ability for cross-reactive antibodies to interfere in immunoassays.

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SARS-CoV-2 spike L452R variant evades cellular immunity and increases infectivity

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Abstract

Many SARS-CoV-2 variants with naturally acquired mutations have emerged. These mutations can affect viral properties such as infectivity and immune resistance. Although the sensitivity of naturally occurring SARS-CoV-2 variants to humoral immunity has been investigated, sensitivity to human leukocyte antigen (HLA)-restricted cellular immunity remains largely unexplored. Here, we demonstrate that two recently emerging mutations in the receptor-binding domain of the SARS-CoV-2 spike protein, L452R (in B.1.427/429 and B.1.617) and Y453F (in B.1.1.298), confer escape from HLA-A24-restricted cellular immunity. These mutations reinforce affinity toward the host entry receptor ACE2. Notably, the L452R mutation increases spike stability, viral infectivity, viral fusogenicity, and thereby promotes viral replication. These data suggest that HLA-restricted cellular immunity potentially affects the evolution of viral phenotypes and that a further threat of the SARS-CoV-2 pandemic is escape from cellular immunity.

› Mol Med Rep. 2021 Aug;24(2):584. doi: 10.3892/mmr.2021.12223. Epub 2021 Jun 16.

SARS-CoV-2 spike protein-induced host inflammatory response signature in human corneal epithelial cells

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Abstract

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), led to an outbreak of viral pneumonia in December 2019. The present study aimed to investigate the host inflammatory response signature-caused by SARS-CoV-2 in human corneal epithelial cells (HCECs). The expression level of angiotensin-converting enzyme 2 (ACE2) in the human cornea was determined via immunofluorescence. *In vitro* experiments were performed in HCECs stimulated with the SARS-CoV-2 spike protein. Moreover, the expression levels of ACE2, IL-8, TNF-α, IL-6, gasdermin D (GSDMD) and IL-1β in HCECs were detected using reverse transcription-quantitative PCR and/or western blotting. It was identified that ACE2 was expressed in normal human corneal epithelium and HCECs cultured *in vitro*. Furthermore, the expression levels of IL-8, TNF-α and IL-6 in HCECs were decreased following SARS-CoV-2 spike protein stimulation, while the expression levels of GSDMD and IL-1β were increased. In conclusion, the present results demonstrated that the SARS-CoV-2 spike protein suppressed the host inflammatory response and induced pyroptosis in HCECs. Therefore, blocking the ACE2 receptor in HCECs may reduce the infection rate of COVID-19.

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Long-lived macrophage reprogramming drives spike protein-mediated inflammasome activation in COVID-19

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Abstract

Innate immunity triggers responsible for viral control or hyperinflammation in COVID-19 are largely unknown. Here we show that the SARS-CoV-2 spike protein (S-protein) primes inflammasome formation and release of mature interleukin-1β (IL-1β) in macrophages derived from COVID-19 patients but not in macrophages from healthy SARS-CoV-2 naïve individuals. Furthermore, longitudinal analyses reveal robust S-protein-driven inflammasome activation in macrophages isolated from convalescent COVID-19 patients, which correlates with distinct epigenetic and gene expression signatures suggesting innate immune memory after recovery from COVID-19. Importantly, we show that S-protein-driven IL-1β secretion from patient-derived macrophages requires non-specific monocyte pre-activation in vivo to trigger NLRP3-inflammasome signaling. Our findings reveal that SARS-CoV-2 infection causes profound and long-lived reprogramming of macrophages resulting in augmented immunogenicity of the SARS-CoV-2 S-protein, a major vaccine antigen and potent driver of adaptive and innate immune signaling.

› Phys Chem Chem Phys. 2021 Jun 17. doi: 10.1039/d1cp01075a. Online ahead of print.

Quantitative analysis of ACE2 binding to coronavirus spike proteins: SARS-CoV-2 *vs.* SARS-CoV and RaTG13

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Abstract

The global outbreak of the COVID-19 pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Bat virus RaTG13 and SARS-CoV are also members of the coronavirus family and SARS-CoV caused a world-wide pandemic in 2003. SARS-CoV-2, SARS-CoV and RaTG13 bind to angiotensin-converting enzyme 2 (ACE2) through their receptor-binding domain (RBD) of the spike protein. SARS-CoV-2 binds ACE2 with a higher binding affinity than SARS-CoV and RaTG13. Here we performed molecular dynamics simulation of these binding complexes and calculated their binding free energies using a computational alanine scanning method. Our MD simulation and hotspot residue analysis showed that the lower binding affinity of SARS-CoV to ACE2 vs. SARS-CoV-2 to ACE2 can be explained by different hotspot interactions in these two systems. We also found that the lower binding affinity of RaTG13 to ACE2 is mainly due to a mutated residue (D501) which resulted in a less favorable complex formation for binding. We also calculated an important mutation of N501Y in SARS-CoV-2 using both alanine scanning calculation and a thermodynamic integration (TI) method. Both calculations confirmed a significant increase of the binding affinity of the N501Y mutant to ACE2 and explained its molecular mechanism. The present work provides an important theoretical basis for understanding the molecular mechanism in coronavirus spike protein binding to human ACE2.

› Am J Physiol Lung Cell Mol Physiol. 2021 Jun 22. doi: 10.1152/ajplung.00223.2021. Online ahead of print.

The SARS-CoV-2 Spike Protein Subunit 1 induces COVID-19-like acute lung injury in K18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells

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Abstract

Acute lung injury (ALI) leading to acute respiratory distress syndrome is the major cause of COVID-19 lethality. Cell entry of SARS-CoV-2 occurs via the interaction between its surface Spike protein (SP) and angiotensin converting enzyme-2 (ACE2). It is unknown if the viral Spike protein alone is capable of altering lung vascular permeability in the lungs or producing lung injury in vivo. To that end, we intratracheally instilled the S1 subunit of SARS-CoV-2 Spike protein (S1SP) in K18-hACE2 transgenic mice that overexpress human ACE2 and examined signs of COVID-19 - associated lung injury 72 hours later. Controls included K18-hACE2 mice that received saline or the intact SP and wild-type (WT) mice that received S1SP. K18-hACE2 mice instilled with S1SP exhibited a decline in body weight, dramatically increased white blood cell and protein concentrations in bronchoalveolar lavage fluid (BALF), upregulation of multiple inflammatory cytokines in BALF and serum, histological evidence of lung injury and activation of STAT3 and NFκB pathways in the lung. K18-hACE2 mice that received either saline or SP exhibited little or no evidence of lung injury. WT mice that received S1SP exhibited a milder form of COVID-19 symptoms, compared to K18-hACE2 mice. Further, S1SP, but not SP, decreased cultured human pulmonary microvascular transendothelial resistance and barrier function. This is the first demonstration of a COVID-19-like response by an essential virus encoded protein by SARS-CoV-2 in vivo. This model of COVID-19-induced ALI may assist in the investigation of new therapeutic approaches for the management of COVID-19 and other coronaviruses.

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SARS-CoV-2 spike protein induces paracrine senescence and leukocyte adhesionin endothelial cells

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Free article

Abstract

Increased mortality in COVID-19 often associates with microvascular complications. We have recently shown that SARS-CoV-2 spike protein promotes an inflammatory cytokine IL-6/IL-6R induced trans-signaling response and alarmin secretion. Virus infected or spike transfected human epithelial cells exhibited an increase in senescence state with the release of senescence associated secretory proteins (SASP) related inflammatory molecules. Introduction of BRD4 inhibitor AZD5153 to senescent epithelial cells reversed this effect and reduced SASP related inflammatory molecule release in TMNK-1 or EA_{hy926} as representative human endothelial cell line, when exposed to cell culture medium (CM) derived from A549 cells expressing SARS-CoV-2 spike protein, also exhibited a senescence phenotype with enhanced p16, p21, SA-β-galactosidase expression, and triggered SASP pathways. Inhibition of IL-6 trans-signaling by Tocilizumab and inhibition of inflammatory receptor signaling by the BTK inhibitor Zanubrutinib, prior to exposure of CM to endothelial cells, inhibited p21 and p16 induction. We also observed an increase in reactive oxygen species (ROS) in A549 spike transfected and endothelial cells exposed to spike transfected CM. ROS generation in endothelial cell lines was reduced after treatment with Tocilizumab and Zanubrutinib. Cellular senescence was associated with an increased level of the endothelial adhesion molecules, VCAM-1 and ICAM-1 with *in vitro* leukocyte attachment potential. Inhibition of senescence or SASP function prevented VCAM-1/ICAM-1 expression and leukocyte attachment. Taken together, we identified that the exposure of human endothelial cells to cell culture supernatant derived from SARS-CoV-2 spike protein expression displayed cellular senescence markers, leading to enhanced leukocyte adhesion. **Importance:** The present study was aimed at examining the underlying mechanism of extrapulmonary manifestations of SARS-CoV-2 spike protein associated pathogenesis, with the notion that infection of the pulmonary epithelium can lead to mediators that drive endothelial dysfunction. We utilized SARS-CoV-2 spike protein expression in cultured cells of human hepatocytes (Huh7.5) and pneumocytes (A549) to generate conditioned culture media (CM). Endothelial cell lines (TMNK-1 or EA_{hy926}) treated with CM exhibited increase in cellular senescence markers by a paracrine mode, and lead to leukocyte adhesion. Overall, the link between these responses in endothelial cell senescence, and a potential contribution to microvascular complication in productively SARS-CoV-2 infected humans is implicated. Furthermore, the use of inhibitors (BTK, IL-6 and BRD4) showed reverse effect in the senescent cells. These results may support the selection of potential adjunct therapeutic modalities to impede SARS-CoV-2 associated pathogenesis.